

FLORIAN KERN ET AL.  
USSN 09/600,564  
REPLY TO OFFICE ACTION DATED MAY 4, 2004  
AMENDMENT OF APRIL 4, 2005

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. to 13. (Canceled)

14. (Previously presented) A method for the identification of T-cell stimulating protein fragments comprising the following steps:

- a) establishing the amino acid sequence of an antigen which is a protein or a peptide;
- b) subdividing the amino acid sequence of said antigen into protein fragments;
- c) synthesizing at least one protein fragment having a length of from 8 to 30 amino acids, or cleaving the amino acid sequence of said antigen into at least one protein fragment having a length of from 8 to 30 amino acids, wherein said protein fragment is a subsequence of the established amino acid sequence of said antigen;
- d) incubating a suspension containing T cells with the protein fragment or

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fragments in different experimental runs;

e) identifying of

(i) at least one T cell cytokine which has been induced by the protein fragment or fragments and synthesized in the T cells, wherein the T cell cytokine or cytokines remain within the cell or are bound to the cell membrane; and/or

(ii) at least one activation marker expressed or expression-enhanced due to the T cell stimulation by the protein fragment or fragments which has been induced or expression-enhanced by the protein fragment or fragments and which is expressed in the T cells, wherein said activation marker can be present within the cell or expressed on the cellular surface;

wherein said T cell cytokine or cytokines or activation markers are identified by flow cytometry; and

f) assigning the experimental runs in which T cells have been stimulated and such stimulation has been recognized by the identification of one or more

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T cell cytokines and/or one or more activation markers, to the amino acid sequence or sequences of said protein fragments which had been incubated with the T cells;

characterized in that the incubation time is sufficiently long so that the protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules present on the cellular surface, said taking up being sufficient when an unambiguous identification of stimulated T cells is possible; and

the incubation time of the suspension containing T cells with the protein fragment or fragments is sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T cells do not occur.

15. (Previously presented) The method for the identification of T-cell stimulating protein fragments according to claim 14, wherein said identification of at least one T cell cytokine or activation marker is made on the individual cell level.

16. (Previously presented) The method for identification of T-cell stimulating protein fragments according to Claim 14, wherein said suspensions containing T cells contain cells which present the protein fragment essentially in a state bound to MHC class I or class II molecules.

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17. (Previously presented) The method for the identification of T-cell stimulating protein fragments according to claim 14, wherein the protein fragment in the class I restricted presentation comprises from 9 to 11 amino acids, and the protein fragment in the Class II restricted presentation comprises at least 11 amino acids.

18. (Previously presented) The method for the identification of T-cell stimulating protein fragments according to claim 14, wherein said suspension containing T cells is a suspension of whole blood, peripheral white blood cells (PWBC), splenocytes, thymocytes, bone marrow, cerebrospinal fluid and/or lymph node cells.

19. (Previously presented) The method for identification of T-cell stimulating protein fragments according to claim 14, wherein said suspension containing T cells is derived from patients to be subjected to therapy, from donors or from animals.

20. (Previously presented) The method for the identification of T-cell stimulating protein fragments according to claim 14, wherein the protein or peptide antigens are derived from multicellular eukaryotes, cells and/or tissues thereof, and cell cultures and/or tissues of donors or patients.

21. (Previously presented) The method for the identification of T-cell stimulating

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protein fragments according to claim 14, wherein the T cell cytokines are of the types interferon- $\gamma$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) or interleukin 2.

22. (Withdrawn) A process for the preparation of a protein fragment/peptide which is T-cell stimulating and whose amino acid sequence or initial amino acid sequence was found by the method for the identification of T-cell stimulating protein fragments according to claim 14, wherein said protein fragment/peptide is prepared by the solid phase method, liquid phase method or by protein biosynthesis in a host.

23. (Withdrawn) The process for the preparation of a protein fragment/peptide according to claim 22, wherein said protein fragment/peptide contains insertions, deletions or substitutions (modifications) wherein one, two, three or more amino acids have been exchanged, deleted or inserted, wherein said modified protein fragment/peptide has essentially the same function with respect to the stimulation of T cells as the non-modified protein fragment/peptide.

24. (Withdrawn) The process for the preparation of a protein fragment/peptide according to claim 22, wherein said protein fragment/peptide contains at least one additional naturally occurring or not naturally occurring amino acid and/or protecting group at the N-terminal and/or C-terminal end (extended modification), wherein the extendedly modified protein fragment/peptide has essentially the same function with respect to the stimulation of T

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cells as the non-modified protein fragment/peptide.

25. (Withdrawn) Method of using of a protein fragment/peptide prepared by the process according to claim 22 for the preparation of a medicament for immune stimulation.

26. (Withdrawn) Method of using a protein fragment/peptide according to claim 25, wherein said immune stimulation is a vaccination or desensitization.

27. (New) A method for identifying T-cell stimulating protein fragments, said method comprising the following steps:

- a) establishing the amino acid sequence of a protein or peptide antigen;
- b) providing one or more protein fragments of said protein or peptide antigen, each of said one or more protein fragments having a length of from 8 to 30 amino acids, and each of said one or more protein fragments being a unique subsequence of the amino acid sequence of the protein or peptide antigen established in step a);
- c) incubating for an incubation time a suspension comprising T cells and the one or more protein fragments;

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- d) identifying by flow cytometry:
  - i) one or more T cell cytokines induced by the one or more protein fragments and synthesized in the T cells, wherein the one or more T cell cytokines remain within the T cells or are bound to the cell membrane of the T cells; and/or
  - ii) one or more activation markers expressed or expression-induced due to stimulation of the T-cells by the one or more protein fragments, wherein the one or more activation markers are expressed in the T cells, and the one or more activation markers are present within the T cells or expressed on the surface of the T cells; and
- e) identifying T-cell stimulating protein fragments by ascertaining which of said one or more protein fragments has caused said one or more T cell cytokines and/or said one or more activation markers to be induced;

wherein the incubation time is sufficiently long that the one or more protein fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules present on the

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surface of the T cells that an unambiguous identification of stimulation of the T cells is possible;  
and

the incubation time is sufficiently short that selection and proliferation of the T cells  
accompanied by specific elimination of particular T cells do not occur.